Collective behavior of kinesin-5 motors during anaphase

Sarah Elaz*, Brian Griffin*, and Maria L. Kilfoil*

*Department of Physics, University of Massachusetts Amherst, Amherst, Massachusetts

Submitted to Proceedings of the National Academy of Sciences of the United States of America

At the individual molecule level, the relationship between applied force and steady-state velocity for processive molecular motors is a fundamental characteristic of the processive motor’s enzyme mechanism itself. For an ensemble of motors, the relationship between applied force and steady-state velocity is not known. Questions arise as to whether motors as part of ensembles in cells do act in coordinated fashion or act individually, and whether they operate close to force limits in the intracellular milieu. We made direct measurements of coherent motions during mitotic division in the nucleus of yeast cells to address these questions. We demonstrate application of a new quantitative method using a model that is germane to single motors to describe the collective behavior of ensembles of motors in the living cell.

molecular motor | motor coordination | live cell transport | processivity

Abbreviations: MT, microtubule; wt, wild type; SPB, spindle pole body

The molecular motor kinesin travels processively along a microtubule in a stepwise manner. During anaphase of mitosis, the class of kinesin motors called kinesin-5 motors drive outward motion of the mitotic spindle, which drives chromosome segregation. During anaphase of mitosis, these motors act coherently, with high processivity and high affinity for MTs, due to a rapidly-effected change in their biochemical state with the global cell state upon the metaphase-to-anaphase transition [1, 2, 3, 4, 5]. The kinesin-5 motors and the spindle mid-zone region, where they have this strong interaction with antiparallel MTs, are illustrated generically in Fig. 1a). The tetrameric kinesin-5 motors exert forces on two antiparallel MTs simultaneously at the spindle mid-zone, comprising an extensile force dipole at the mid-zone. Collectively, the motors drive spindle pole separation during anaphase.

Introduction

Recently, both types of kinesin-5 tetramer motors from yeast have been shown to exhibit a remarkable property uncommon among kinesin motors: individual motors sometimes switch their direction of travel on a MT when multiple motors crosslink two anti-parallel MTs [6, 7, 8]. Minimal systems that can recapitulate this internal tension generated by these motors, or their transport properties, on microtubules in vitro have been developed that allow study of the motor activity in purified form or in cell extracts [3, 7, 8]; however, in these systems it is difficult to control the MT polarity and the the phosphorylation state of the molecular motors. Other bottom-up approaches have successfully re-created antiparallel microtubules and the motion of single motors was studied in this in vitro milieu [6]. Bottom-up approaches have proven invaluable for understanding properties of individual molecular motors, but it has nevertheless proven challenging in in vitro systems to study ensembles of motors naturally and controllably, without engineering them to be physically linked [9].

This latter approach is powerful and has yielded tantalizing new insights into potential collective behavior of motors. For example, tracking of two kinesin-1 molecules organized on a linear scaffold at fixed 50 nm separation revealed that while two motors produce longer average run lengths than single kinesins, the system effectively behaves as though a single-motor attachment state dominates motility [10].

Here, we report on measurements of spindle elongation in both normal (wt) budding yeast cells, and cells engineered to express only one of the two yeast kinesin-5 motors (Cin8 or Kip1). By analyzing the individual stepwise movements according to the directionality of the movements, we use this data to elucidate the load-bearing capacity of the mitotic motors during anaphase chromosome segregation. These findings may necessitate revisions of our understanding of how teams of kinesin-5 motors function on the spindle.

Results

We have used the model eukaryote Saccharomyces cerevisiae to investigate the motor-driven motion of the spindle in detail. To report on the motor-driven motion, we use the separation of the spindle poles in 3D, which we term $L(t)$. This measure reports the aggregate motor+p+directed kinesin-5 activity, since kinesin-5 dominates the outward (extensile) motion of the mitotic spindle during budding yeast anaphase. To quantify spindle length, we tagged a spindle-pole body protein with a fluorescent protein and used a fast scanning multi-beam...

Significance

Motor proteins or molecular motors are enzymatic molecules that actively participate in all major biological processes such as cellular transport, cell division, transfer of genetic information, synthesis of proteins, cell motility and signaling. Biological molecular motors have been intensively studied in recent years and currently the single-molecule dynamics of motor proteins is well described. However, many fundamental aspects of transport are poorly understood. Although the properties of individual molecules are very useful, in biological systems motor proteins typically function in teams. To develop understanding of the collective behavior of molecular motors in living cells, we made direct measurements of coherent motions during mitotic division in the nucleus of yeast cells that we made minimal with respect to the molecular motors that drive this process. Our approach demonstrates a new quantitative method using models that are germane to single motors, to describe some of the most interesting and informative properties of motors working together to organize and effect the segregation of chromosomes in cells.

Reserved for Publication Footnotes
confocal microscope to carry out three-dimensional time-lapse imaging (see Materials and Methods).

The data for normal cells is shown in Fig. 1. Each shade of blue represents a different cell. Plotted together, the data show that the cells all exhibit approximately the same time evolution of the anaphase spindle length, particularly during the early, rapid phase of extension. During late anaphase, the rate of spindle elongation shows greater variation from cell to cell.

These data show that the separation between the two poles in anaphase serves as a good reaction coordinate to follow the kinetics of kinesin-5 motors which drive spindle dynamics in cells. They confirm prior observations in budding yeast cells [11, 12]. To compare the data from all the cells directly, we took the anaphase velocity for each cell to be the local tangent to $L(t)$ during anaphase (see Fig. 1 b). We plot the anaphase velocity for all these cells together in Fig. 1 c). The data describe a common curve; no scaling was performed. The prior work described the spindle pole separation rate as switching from one, high speed during early anaphase, to another, lower speed during mid- to late-anaphase [11, 12]. However, the earlier studies lacked the improved resolution afforded by the high temporal resolution and high position resolution of our measurements, and subtle changes in shape were not distinguished within experimental error. Our data reveals that the velocity changes continuously. The change in velocity suggests a force-velocity relation. Many prior single molecule studies demonstrate that individual kinesin molecular motors across different kinesin classes slow down when subjected to a load that opposes their motion [13, 14, 15, 16].

In eukaryotic cells, protein linkers that act to catenate the sister chromatids during metaphase, are removed at the transition to anaphase [17, 2]. One possible explanation for the early rise in the anaphase velocity in our data is that some incompressible time is required for the removal of these protein linkers. During this time, sister chromatids are pulled apart with increasingly greater ease, as their protein connections are removed.

The velocity reaches a maximum and then begins to drop continuously. This again suggests a force-velocity relationship of motors: Our intuition from single-molecule motor studies suggests there must be a force that is causing this slowing down. The data here imply that additional load slows the motors down some short time after the motors begin to drive the spindle, and that the load thereafter continually increases, until late anaphase when it saturates. A continually increasing load must be coming from the DNA. Although MTs and the mitotic spindle are highly dynamic structures. Throughout mid- to late-anaphase the spindle length is kept remarkably constant, due to properties built into the chemistry of the MT, motor protein, and cross-linker components, and to their regulation by the cell [18, 19, 4, 20, 21]. It is therefore within reason that the component motor numbers at the spindle mid-zone should remain approximately constant during this time.

The possibility that an additional load slows the motors down is also supported by data shown in Supplementary Information, Fig. S1, which demonstrates that individual kinesin motors (Cin8 and Kip1) make the remaining one essential for cell viability. For example, perturbations in either of the kinesin-5 mitotic motors (Cin8 and Kip1) make the remaining one essential for cell proliferation. Different organisms may use different additional functions to convert a minimal system into a full one.

Minimal Ensemble Kinesin-5 Studies. Detailed analysis of the dynamics of minimal systems in budding yeast and other organisms should illuminate the behaviors of individual motor protein ensembles that perform the driving of the spindle solely. Knowledge of the detailed dynamics, interpreted with a microscopic mechanochemical model, can be used to place constraints on the underlying mechanical system. We carried out experiments to track the spindle poles in the same strain of budding yeast cells now depleted of Cin8, and separately of Kip1. Analysis of spindle dynamics was carried out for a population of each cell type. The data for normal cells is shown in Fig. 1. Each shade of blue represents a different cell type. Fig. 2 a) and b) shows $L(t)$ for cells engineered to express only one of the two yeast kinesin-5 motors: Cin8 ($kip^{−1} \Delta$; a), or Kip1 ($cin8^{−1} \Delta$, b)). The $kip^{−1} \Delta$, Cin8-bearing cells appear coherent over the entire regime. By contrast, in the other knockout, $cin8^{−1} \Delta$, there is significant variation in the rate of spindle elongation, and $L(t)$ appears noisier for $cin8^{−1} \Delta$ cells than for $kip^{−1} \Delta$ or $wt$ cells over the entire regime. These observations suggest that regulation of coordinated motor activity for Kip1 as compared to Cin8.

Despite this heterogeneity within the population of cells of type $cin8^{−1} \Delta$, and across the populations (kinesin-5 knockouts and $wt$), we found that, surprisingly, $L(t)$ for all these anaphase cells could be scaled vertically to collapse onto a common curve (Fig. 2 c). To construct this collapse of the data, we compare the behavior of all the cells directly, the separation curve $L(t)$ for each cell is shifted to the time when it begins anaphase, which is strictly arbitrary in any case; and shifted in length relative to its metaphase length. The residual, relative length is then scaled by a multiplicative factor that achieves best collapse of the scaled curve for that cell onto the unscaled data of an arbitrarily-chosen $wt$ cell. Hence $L′(t) = S_i(L(t) - L_{meta,i})$ describes the transformation for each cell $i$. The entire $L(t)$ curve for each cell is transformed in this way, and the resulting scaled curves are plotted together in Fig. 2 c). Alternative ways of scaling the data were explored, and this method gave the greatest agreement between the curves, thereby revealing the underlying scaling: the difference from cell to cell is the speed with which the spindle is elongated in anaphase. The data collapse demonstrates that the anaphase spindle length at any one cell, can be scaled according to the same functional form for all the cells, despite the broad range of anaphase speeds across the knockout and $wt$ cells. The multiplicative scale factors $S_i$ required to collapse the data together quantify the broad differences in the spindle elongation rates between the individual cells. The distribution of $S_i$ for each population is plotted in Fig. 2 d). For the $kip^{−1} \Delta$ population, these scale factors were narrowly distributed and slightly greater than 1, while for the $cin8^{−1} \Delta$ population the scale factors ranged from 2 to 5.
Detailed examination of the $L(t)$ trajectories of the cin8Δ cells reveals traces of a few tens of seconds duration and hundreds of nm in length, during which sustained spindle elongation could be detected, resembling coordinated, processive motion at constant rate. These tracts alternated with tracts of sustained motion in the reverse direction of tens of nm in length. Evidently, the Kip1 motor ensemble switches the direction of its driving of the spindle elongation during chromosome segregation.

In each cin8Δ cell, the $L(t)$ data exhibited this directional switching. The speed of the “forward” (extensile, +end-directed) motions is constant as time evolves (Fig. 3). From the data, the ratio of backward stepping rate to forward stepping rate (parameterized as the duty ratio of negative stepping) varies from cell to cell, and within each cell, increases with time during anaphase. Strikingly, this speed is similar across the cin8Δ population, to within <25% deviation (Fig. 3 inset), despite the factors of 2 to 5 differences in the overall spindle separation rate across this population. Conversely, the speed of the reverse motions is not constant within each cell.

These observations, together with the fact that during anaphase the motors must work against the chromosome load, suggest a microscopic model in which the rate of outward stepping is insensitive to the external force.

Discussion

Discrete stochastic models are already in wide use to describe single molecular motor mechanics [22], starting with a single-state “biased diffusion” model (strictly speaking, the model was initially intended for statistics of ensembles of motor proteins) [23, 24], in turn based a model of one-dimensional hopping on a lattice developed by Derrida [25]. In such a model, the stepping of the molecular motor is stochastic: the forward and reverse stepping rates are probability functions, $k_+$ and $k_-$, and in the zero-load situation are related through the energy of ATP hydrolysis: $r = k_+/k_- = e^{\Delta G/k_B T}$. The negative free energy $\Delta G$ available for a motor to do work via the hydrolysis of ATP (or other nucleotides) may be found from biochemical studies. Under physiological conditions, $|\Delta G| \approx 20k_B T$ [26]. The motor molecules thus move primarily in the forward direction and only occasionally in the backward direction. In the zero-load situation, the steady state velocity obtained in this model for a lattice with the periodicity of the step size, as MTs posses, is the very simple result $v(t) = u_0(k_+ - k_-)$ [25].

The load situation is embodied by allowing either $k_+$ or $k_-$ (or both) to couple to the external force. The unitary step size should be that of the MT lattice and is thereby independent of load. Schnitzer et al. [27] proposed an exponential dependence of the change in stepping duty ratio on the applied force, $r' = r e^{u_0/k_B T}$, where $r$ refers to the zero load situation and $u_0$ is the step length. One may then view the imposition of a load $f$ most simply as adding a term in the free energy: $r' = r - f k_0/k_+$. Which of the probability functions ($k_+, k_-$) couple to the external force will determine the shape of the force-velocity curve, but irrespective of that detail, larger force increases $r'$. The force at which the motor can no longer do net mechanical work against the load, the stall force $f_s$, is characterized by vanishing velocity, and $r'(f_s) = 1$, hence $f_s = (\Delta G)/u_0$.

We observed a general decrease in kinesin-5-driven spindle extension velocity with increasing time in each cell. The implication of the model applied to our cin8Δ $L(t)$ anaphase data is that the mechanical work exerted by the Kip1 motor ensemble against the external load increases with time for each cell; and varies from cell to cell. This is likely to be true in all the cells during anaphase – i.e. of the other minimal system bearing only the Cin8Δ envelope, or the full system bearing the full complement of kinesin-5 motors – but the Kip1 ensemble displays the more marginal behavior under the load. This could be due to fewer Kip1 motors active at the spindle mid-zone compared to Cin8Δ; differences in affinity for the spindle; or differences in the degree of coordination of collective behavior between these two kinesin-5 motors.

Analysis of Velocity Curves. Our observations lead us to treat $k_+$ as load-independent for the Kip1 motors, and $k_-$ as the probability distribution that couples to the external force. Then $v(t) = u_0k_+ (1 - r'(t))$, $k_+$ is known from the analysis for each cell, and $u_0$ is the fixed lattice distance for kinesin stepping on MTs, 8 nm. We obtain $v(t)$ in each cell by faithfully fitting the overall evolution of $L(t)$ with a smooth curve (a lower-order polynomial), then taking the gradient of that curve to be $v(t)$. This procedure, demonstrated on the data for one representative cin8Δ cell is shown in Fig. 4 inset, yields a continuous and robust measure of $r'$ as a function of time. The model can be re-written in terms of the measured, observable quantities in each cell, $k_+$ and $v(t)$, as $f(t) - f_s = k_B T/u_0 \ln(r'(t))$ with $r'(t) = 1 - v(t)/(u_0 k_+)$. If this model correctly describes our data, the velocity (alternatively, the stepping rates) yields an approximation to the force $f(t)$ exerted by the Kip1 motor ensemble in each cin8Δ cell, to within an additive constant, the stall force. The model was fitted to the entire set of raw (unscaled) cin8Δ cell data. $f - f_s$ from the model was obtained in this way for each cin8Δ cell, using $k_B T = 4.1 \text{pN nm}$ (plotted in Fig. 4). Since the dynamic range of $f_s - f(t)$ from this analysis never exceeds ~2.5 pN, the data suggests that the stall force – a property of the Kip1 on the antiparallel MTs in this cellular milieu – is ~2.5 pN.

Comparison with Other Force Measurements. Earlier measurements using differing approaches provide similar estimates of kinesin-5 parameters. The simple stepping cycle yields results consistent with all the other known data for the full length kinesin-5 tetramer. Single molecule optical trapping experiments on a full-length Eg5 (the kinesin-5 from Xenopus) found that the force produced by individual motors, with their unique cellular role, to have a low efficiency may from hydrolysis of one ATP molecule. This efficiency is somewhat lower than typical values discovered for conventional, dimer kinesin (40%–60%, [30, 13]), and is consistent with the values obtained for myosin minifilaments (12%–42% [31, 32]). As implied by Korobeev et al. [28] , for kinesin-5, efficiency may not be the appropriate performance metric. It may be optimal to be run by individual cells to move the load to a low force limit and to switch direction, rather than stall, close to that limit, when they act collectively at the spindle mid-zone.

Future work should enable testing of other motor-driven force-generating processes in living cells and other minimal systems, including in vitro systems, using this approach – importantly, so long as the observable quantities include a measure of the aggregate motor-driven motion, and not (or not solely) the motion of the motors themselves, which does not.
Such studies will serve as calibration for in vivo studies, and to place constraints on the regimes in which we can describe molecule motor collective behavior using models that are germane to single motors. This is a novel perspective for motors in living cells, that opens up the interpretation of tracking statistics from motions of motor ensembles exhibiting bi-directional motion in experiments. If it turns out to hold generally, this approach allows us to bridge the gap between refined single-molecule experiments/models, and collective behavior of molecular motors in their cellular milieu, which are recovered in particular conditions, to begin to understand the principles of collective motor coordination at antiparallel MT lattices involved in major cellular roles.

While the model used has only one kinetic transition, whereas kinesin-1 single molecule data has revealed multiple steps and substeps in that dimer motor [35], there is not yet been obtained the corresponding statistics from single molecule data for full-length kinesin-5 that would enable testing of this. These molecular motors may, in future, with such single molecule data for analysis, be shown to have multiple motors. The correct combination will be motor operating in ensembles on antiparallel MT arrays, not isolated motors on a head. The model we have used here was employed for the early force-velocity response descriptions of single molecule data from conventional kinesin, and has successfully described the data for myosin stepping [33]. In future work, even further enhancement of the sampling rate used here, by a factor of 2-5, might be expected by employing further improvements to fluorescent probes in the experiments, at which point the data may reveal features of two-state behavior if it is present.

Materials and Methods

Cell Preparation.

The S. cerevisiae strains used were BY4741 genetic background, derivatives of the S288C strain; expressing Spc42-GFP or Spc42-tomato. All cells were cultured and imaged in synthetic complete (SC) minimal medium, chosen for growth out of stationary phase.

Cell Preparation.

S. cerevisiae strains used were BY4741 genetic background, derivatives of the S288C strain; expressing Spc42-GFP or Spc42-tomato. All cells were cultured and imaged in synthetic complete (SC) minimal medium, chosen for growth out of stationary phase.

Acknowledgments.

Financial support was provided by the University of Massachusetts Amherst.


36. Y. Gao and M. Kilfoil, Accurate detection and complete tracking of large populations of features in three dimensions, Optics Express 17 (2009), pp. 4685.
**Figure legends**

**a)** Operation of a basic unit of the mitotic spindle motor-driven machinery and forces. During metaphase and anaphase, chromosomes are attached to the spindle for segregation and pushed apart by the kinesin-5 motor driving.

**b)** Live cell data of spindle length $L(t)$ vs. time (a reaction coordinate for anaphase) for all wild type cells that passed through anaphase. Anaphase velocity $v(t)$ is obtained from $dL(t)/dt$. Each gray line represents one cell and tracing out a common curve. $t = 0$ is taken to be the start of anaphase, and $t_{ana}$ the time relative to this, for each cell. Blue solid line is the average wt $v(t)$.

**c)** Live cell data of spindle length $L(t)$ vs. time for all wild type cells from b), each gray line representing one cell and tracing out a common curve. $t = 0$ is taken to be the start of anaphase, and $t_{ana}$ the time relative to this, for each cell. Blue solid line is the average wt $v(t)$.  

---

*Fig. 1.* a) Operation of a basic unit of the mitotic spindle motor-driven machinery and forces. During metaphase and anaphase, chromosomes are attached to the spindle for segregation and pushed apart by the kinesin-5 motor driving. b) Live cell data of spindle length $L(t)$ vs. time (a reaction coordinate for anaphase) for all wild type cells that passed through anaphase. Anaphase velocity $v(t)$ is obtained from $dL(t)/dt$. c) $v(t)$ for all 28 wt cells from b), each gray line representing one cell and tracing out a common curve. $t = 0$ is taken to be the start of anaphase, and $t_{ana}$ the time relative to this, for each cell. Blue solid line is the average wt $v(t)$. 

---

*www.pnas.org/cgi/doi/10.1073/pnas.0709640104*
Fig. 2.  a-b) Live cell data of spindle length $L(t)$ vs. time for each anaphase cell in populations in which the collective activity of only one type of the yeast kinesin-5 motors drives the motion: a) the $kip_1\Delta$ population (each shade of orange represents a single cell), and b) the $cin_8\Delta$ population (each shade of green represents a single cell). c) The reaction coordinate for each cell is scaled vertically to collapse the data from all anaphase cells in all 3 populations onto a single curve. d) Probability distributions of the multiplicative factors required to scale the data in c), plotted for each of the 3 (wt, $kip_1\Delta$, and $cin_8\Delta$) populations.

Fig. 3. $L(t)$ vs. time through anaphase for one cell bearing only the Kip1 ensemble. From the forward stepping traces, the (+)-end directed processive velocity was obtained. Inset: the aggregate forward stepping traces for all the cells in the $cin_8\Delta$ population. The (+)-end directed processive velocity was similar from cell to cell within 25%.
Fig. 4. The force obtained for each cell using the one-state motor case discussed in the text, obtained to within an additive constant, the stall force $f_{st} = |\Delta G|/u_0$. Using this analysis, all the kip1-driven cells approach $f_{st}$. Inset: Overall $L(t)$ profile (black solid line) describing the spindle length evolution, obtained by fitting the $L(t)$ data to a low order polynomial. $v_{av}(t)$ is extracted as the gradient of this fit.